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(54) Title: HYDROPHILISED SOLID SURFACE, METHOD FOR THE PRODUCTION THEREOF, AND AGENT THEREFOR

(57) Abstract

A hydrophilised solid surface having a biopolymer-repellent hydrophilic outer layer is produced by causing a solid surface with anionic groups capable of reacting with amino groups, to react with a polyethylene imine which is partly substituted by hydrophilic nonionic polymer chains having a molecular weight of 400-200,000. In this manner, it is possible to obtain a solid surface having a hydrophilic outer layer consisting of nonionic polymer chains which at one end are covalently bonded to a polyethylene imine which in turn is bonded to the solid surface via anionic groups capable of reacting with the amino groups in the polyethylene imine, the weight of the nonionic polymer chains being at least 50 % of the weight of the polyethylene imine. Such hydrophilised solid surfaces exhibit low spontaneous adsorption of biomolecules.

+ DESIGNATIONS OF "SU"

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HYDROPHILISED SOLID SURFACE, METHOD FOR THE PRODUCTION
THEREOF, AND AGENT THEREFOR

The present invention relates to a hydrophilised surface having a high density of hydrophilic polymer chains, whereby biofouling is notably reduced.

Biofouling, i.e. the undesired adsorption of proteins and other biopolymers, is a well-known problem in many biotechnical and mediotectical contexts. A primary adhesion of soluble polymers frequently causes cells, cell debris, bacteria, virus etc. to be bonded to the surface, resulting in the clogging of membranes for e.g. ultra- and microfiltration, impaired performance of chromatographic columns, bacterial infections in conjunction with the use of catheters, etc.

It is generally assumed that the adhesion of cells and lower organisms is always preceded by the adsorption of biopolymers, primarily proteins, and by minimising the protein bond to a surface it would thus be possible to avoid biofouling.

The basic problem is the same for all surfaces, both organic and inorganic, even though it may be more pronounced for some materials than for others. A general rule is that the adsorption of polymers to solid surfaces from aqueous solutions is controlled by hydrophobic interactions and electrostatic forces of attraction.

One way, and a very interesting one, to prevent biofouling, is to coat the surface with a film of a hydrophilic uncharged material. Examples of such materials are polysaccharides, such as dextran, and polyethylene glycol derivatives. To prevent the far-reaching hydrophobic forces of attraction, the layer should not be too thin, preferably not below 10 nm. Furthermore, it has proved to be advantageous if the material contains blocks having a high mobility out to the bulk phase. In respect of protein repellency, polyethylene glycols thus are anchored far more effectively at one end to a polymer matrix than are

polyethylene glycols having both ends bonded to the matrix.

One way of attaching polyethylene glycol tails to a solid polymer surface is to first subject the surface to so-called acidic etching, then to adsorb a cationic polymer, polyethylene imine, to the surface, and finally to react a reactive polyethylene glycol derivative to available amino groups in the polyethylene imine layer. This technique is described in "Prog. Colloid Polym. Sci.", 74, 113-119 (1987). The acidic etching which is carried out in potassium permanganate in concentrated sulphuric acid, results in the formation of carboxylic acid and sulphonic acid groups as well as sulphuric acid esters on the surface. To this highly negatively charged polymer surface, the cationic polyethylene imine is extremely strongly bonded by electrostatic forces. Besides, it is likely that salt formations between amino groups in the polyethylene imine and carboxylated sulphonate groups on the surface are gradually transformed into amide bonds upon drying, whereby an even more stable anchoring of the polyethylene imine to the surface is obtained.

Even though hydrophilised surfaces produced by the technique described in the above paper give an improved repellency of biopolymers, the adsorption thereof is still unacceptably high for most applications.

According to the present invention, it has now proved possible to obtain an even stronger bond of hydrophilic polymer chains to the solid surface than is obtainable with the method previously described in which polyethylene glycol is bonded to a surface made amino-functional by adsorption of polyethylene imine. As a consequence of the more efficient grafting of hydrophilic polymer chains, the method of the present invention gives a far better protein repellency from the surface so that biofouling is effectively prevented. According to the invention, a solid surface having a hydrophilic outer layer of hydrophilic polymer chains is produced by causing a solid surface with

anionic groups capable of reacting with amino groups, to react with a polyethylene imine, the amino groups of which are partly substituted by hydrophilic nonionic polymer chains, the weight of the hydrophilic polymer chains being at least 50% of the weight of the polyethylene imine. Preferably, the polymer chains have such a molecular weight and are present in such numbers that the hydrophilic outer chain has a thickness of at least 100 Å. The surprising effect is believed to be due to the fact that it is difficult to bond hydrophilic polymers in a closely packed arrangement to a surface since the individual hydrophilic polymer molecules repel each other. The same repellency occurs when polyethylene imine is reacted with hydrophilic polymers in solution. However, by first producing a polyethylene imine compound with hydrophilic polymer tails in solution and then allowing this graft copolymer to adsorb to the negatively charged surface, the hydrophilic polymer chains will be forced over to the water side. The density and the thickness of the hydrophilic layer formed by the hydrophilic polymer chains is increased considerably as compared to prior art technique. With this approach, the method of the invention is a method by which hydrophilic polymer chains are more closely packed on the surface than is energetically advantageous with respect to the interaction between the repellent hydrophilic polymer chains. However, this interaction is counterbalanced in that the electrostatic attraction between the polyethylene imine and the surface is energetically stronger than the repellency between the hydrophilic polymer chains when these are compelled to pack themselves more closely.

The polyethylene imine with the hydrophilic polymer chains consists of a polyethylene imine skeleton having a molecular weight of 10,000-1,000,000, preferably from 50,000 to 500,000, which to at least 90% by weight is built up of the units $-C_2H_4NH-$, $-C_2H_4N<$ and $-C_2H_4NH_2$ in which preferably less than 20% of the reactive hydrogens of the imino and amino groups are substituted by hydro-

philic polymer chains and, optionally, other substituents, such as alkyl groups, hydroxyl group-containing groups, amide-containing groups, or functional groups utilised upon grafting of the hydrophilic polymer chains. The

5 hydrophilic polymer chains suitably derive from nonionic polymers, such polyethylene glycol or randomly distributed or block-distributed polyalkylene glycols between ethylene oxide and alkylene oxides having 3-4 carbon atoms, or tetrahydrofuran. Other groups of suitable polymers are

10 adducts of ethylene oxide, optionally in combination with higher alkylene oxides or tetrahydrofuran, with a dihydroxy or polyhydroxy compound, such as glycerol and pentaerythritol. Polysaccharides, such as dextran and starch; cellulose ethers, such as methyl cellulose, methyl hydroxypropyl cellulose, or ethyl hydroxyethyl cellulose; and polyvinyl

15 alcohol are other suitable hydrophilic polymers. The hydrophilic polymer chains are water-soluble, and their molecular weight usually is from 400 to 200,000, preferably from 1,000 to 100,000.

20 According to the invention, the polyethylene imine with grafted hydrophilic polymer chains can be produced by causing the polyethylene imine to react with a reactive hydrophilic polymer in a water-based solution. The ratio of reactive amino groups in the polyethylene imine to

25 reactive end groups in the hydrophilic polymer is adjusted such that the hydrophilic polymer is preferably bonded via its one end only. Examples of reactive hydrophilic polymer derivatives are such derivatives as contain epoxides, aldehydes, sulphonic acid esters, such as tresylate, mesylate and tosylate, cyanuric chlorides, carbonyl imidazoles

30 and active carboxylic acid esters. After the reaction between the hydrophilic polymer derivative and the polyethylene imine has been terminated, remaining reactive groups at the ends of the hydrophilic polymer chains can

35 be removed by reaction with, for example, 2-mercaptoethanol and 2-aminoethanol. A different way of attaching hydrophilic polymer chains to the polyethylene imine is to

provide the latter with reactive groups capable of reacting with the hydroxyl groups of the hydrophilic polymer.

Another alternative way of attaching hydrophilic polymer chains to the polyethylene imine is to add ethylene oxide to the latter, optionally in combination with propylene or butylene oxide or tetrahydrofuran.

The resulting water-soluble polyethylene imine derivative is then caused to adsorb to a negatively charged solid surface. Examples of suitable surfaces are those which have a natural negative net charge, such as silica and glass, or those in which the negative charges have been generated by chemical or physical means. Negative charges can be induced on organic polymer surfaces, for example by acidic etching, i.e. a treatment with potassium permanganate in concentrated sulphuric acid, or by plasma- or radiation-induced grafting of, for example, acrylic acid or methacrylic acid. Examples of organic polymers suitable for this purpose are polystyrene, polyvinyl chloride, polyethylene, polymethyl methacrylate, polycarbonate, polysulfone and cellulose acetate. The invention will be further illustrated by the following Examples.

Example 1

A polystyrene plate having the dimensions 2 x 1 cm was washed in 70% ethanol for 3 min. in an ultrasonic bath. The surface of the sample was air-dried and then oxidised for 30 sec. in $\text{KMnO}_4/\text{H}_2\text{SO}_4$ so that carboxylic acid and sulphonic acid groups were formed. The polystyrene plate was thoroughly rinsed in water. 5 g of a polyethylene glycol diepoxide of molecular weight 4,000 was dissolved in 45 ml carbonate buffer of pH 9.5, whereupon 0.43 g polyethylene imine (Polymin SN) of molecular weight 500,000 was added, and the mixture was allowed to react for 3 h at 45°C under agitation. The polystyrene plate thus treated was placed in the solution containing the polyethylene imine derivative which was adsorbed to the oxidised polystyrene surface for 2 h at 40°C.

For comparison, a polystyrene plate activated in the above manner was coated with the polyethylene imine from a 10% solution for 2 h at 40°C and pH 9.5. The resulting polyethylene imine-coated polystyrene surface was then
 5 repeatedly rinsed with water and allowed to react with a 10% solution of the polyethylene glycol-diepoxide for 20 h at 40°C and pH 9.5.

The thickness of the nonionic hydrophilic layer was evaluated by ellipsometry, the dimensions of both the
 10 polyethylene glycol-polyethylene imine layer and of the polyethylene imine layer alone being determined. The thickness of the polyethylene glycol layer was obtained by subtracting the latter value from the former.

The hydrophilisation effect was evaluated also by
 15 protein adsorption measurements by so-called ELISA technique. The amount of adsorbed protein is proportional to the adsorbency at 490 nm.

Table 1

20	<u>Product</u>	<u>Layer thick- ness (nm)</u>	<u>OD 495 nm</u>	<u>Weight ratio PEG/PEI, %</u>
	Untreated polystyrene	1.254	-	-
25	Polystyrene hydro- philised according to the invention	0.107	42	87
	Polystyrene hydro- philised according to the comparison	0.500	1.6	13

30 PEG = polyethylene glycol; PEI = polyethylene imine

The above results show that the polyethylene glycol layer on the surface treated according to the invention reaches far into the aqueous phase, whereas only a thin
 35 layer in which the chains presumably are lying against the solid surface, is formed on the comparison product. The Table also shows that the hydrophilised surface according

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to the invention has a far high r protein repellency than the comparison product.

Example 2

5 Pieces of LD polyethylene, PVC and polystyrene were cleaned in 70% ethanol and sulphated with $\text{KMnO}_4/\text{H}_2\text{SO}_4$ according to Example 1. A branched polyethylene glycol was produced by adding 320 mol ethylene oxide to di-tri-
10 methylol propane and made reactive by reaction with cyanuric chloride. 5 g of the resulting product was dissolved in 45 ml carbonate buffer (pH 9.5), 0.12 g polyethylene imine was added, and the mixture was allowed to react for 3 h at 45°C. Then the resulting polyethylene imine adduct
15 for 2 h at 40°C, whereupon the surfaces were thoroughly rinsed with deionised water.

For comparison, a hydrophilised product was produced by adsorbing polyethelene imine in the form of a 5% solution in carbonate buffer (pH 9.5) to the activated polymer
20 surfaces for 10 min., whereupon the above-mentioned branched polyethylene glycol derivative was reacted with the amino-functional surfaces in the form of a 10% solution for 5 h at 40°C. Finally, the pieces were thoroughly rinsed with deionised water. The test was evaluated with
25 ESCA, and the following results were obtained.

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Table 2

	<u>Product</u>	<u>O/C</u>	<u>N/C</u>	<u>C-O/C-C</u>	<u>Weight ratio PEG/PEI, %</u>
	LD-PE according to the invention	0.43	0.04	3.92	85
5	LD-PE according to the comparison	0.34	0.06	2.43	12
	PVC according to the invention	0.38	0.07	3.82	78
	PVC according to the comparison	0.29	0.11	1.71	10
10	PS according to the invention	0.41	0.06	3.55	90
	PS according to the comparison	0.34	0.15	1.55	16

15 ~~PEG = polyethylene glycol; PEI = polyethylene imine~~

It appears from the Table that the products according to the invention have a far better layer of polyethylene glycol chains than the product produced according to prior art technique. Especially interesting are the values in the column C-O/C-C which indicate the ratios between the carbon atoms in the polyethylene glycol chain and the carbon atoms in the respective LD-PE, PVC and PS surfaces. A higher value thus indicates a better surface coverage.

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Example 3

Pieces of polyethylene (PE) and polyvinyl chloride (PVC) were washed in 70% ethanol and grafted with crotonic acid under radiation with UV light of wavelength 350 nm in the presence of benzophenone as initiator for the introduction of carboxyl groups. A 10% solution of polyethylene glycol-bis(carbonyl imidazole) of molecular weight 4,000 was allowed to react with 0.24% polyethylene imine at pH 8.0 for 3 h. The carboxyl-functional pieces were treated with the polyethylene imine derivative for 2 h at 40°C, the derivatives being irreversibly adsorbed to the surfaces.

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For comparison, test surfaces of polyethylene and polyvinyl choride were prepared. The activated surfaces were allowed to react with polyethylene imine in the form of a 10% solution for 2 h at 40°C and pH 9.5. The surfaces
5 were repeatedly rinsed with water and allowed to react with a 9% solution of polyethylene glycol bis(carbonyl imidazole) of molecular weight 4,000 for 8 h at 40°C and pH 8.0. The hydrophilisation effect was evaluated by protein adsorption meausurements according to so-called
10 ELISA technique. The results were as follows.

Table 3

	<u>Product</u>	<u>OD 490 nm</u>	<u>Weight ratio PEG/PEI, %</u>
15	PE according to the invention	0.057	81
	PE according to the comparison	0.312	11
	PVC according to the invention	0.3	71
	PVC according to the comparison	1.005	9

20 PEG = polyethylene glycol; PEI = polyethylene imine

The above results show a far better protein repellency when the hydrophilisation had been carried out according to the invention.

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Example 4

A polystyrene plate having the dimensions 6 x 1 cm was activated with $\text{KMnO}_4/\text{H}_2\text{SO}_4$, as described in Example 1. A 2% aqueous solution of the nonionic polysaccharide ethyl
30 hydroxyethyl cellulose was treated with 0.3 M NaIO_4 for 2 h at 20°C, aldehyde groups being generated on the polysaccharide. To 50 ml of the polysaccharide solution, 0.4 g of a 30% solution of polyethylene imine of molecular weight 500,000, and 0.2 g sodium cyanoborohydride were
35 add d. After reaction for 2 h at 20°C and pH 7.0, the activated polystyrene surface was placed in the solution,

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pH was raised to 9.5, and the bonding reaction was allowed to proceed for 2 h at 40°C.

The thickness of the nonionic hydrophilic layer as well as the protein adsorption were evaluated as in Example 1. The Table below clearly indicates that the polysaccharide layer on the surface hydrophilised in accordance with the invention reaches far into the solution and provides a highly effective protein repellency.

10 Table 4

<u>Product</u>	<u>Layer thick- ness (nm)</u>	<u>OD 495 nm</u>	<u>Weight ratio PEG/PEI, %</u>
Untreated polystyrene	-	1.390	-
15 Polystyrene hydro- philised according to the invention	23	0.095	180%

EHEC = ethyl hydroxyethyl cellulose; PEI = polyethyl imine

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CLAIMS

1. A method of producing a solid surface having a
5 biopolymer-repellent hydrophilic outer layer of hydro-
philic polymer chains, c h a r a c t e r i s e d in that
a solid surface with anionic groups capable of reacting
with amino groups is caused to react with a polyethylene
imine partly substituted by hydrophilic nonionic polymer
10 chains having a molecular weight of 400-200,000.

2. Method as claimed in claim 1, c h a r a c t e r -
i s e d in that the weight of the hydrophilic nonionic
polymer chains is at least 50% of the weight of the poly-
ethylene imine.

15 3. Method as claimed in claim 1 or 2, c h a r a c -
t e r i s e d in that the nonionic hydrophilic polymer
chains are at least partly hydrophilic polyalkylene glycol
chains deriving from ethylene oxide or from propylene
oxide, butylene oxide and/or tetrahydrofuran distributed
20 randomly or in blocks.

4. Method as claimed in claim 1 or 2, c h a r a c -
t e r i s e d in that the nonionic hydrophilic polymer
chains are derived from water-soluble nonionic cellulose
ethers.

25 5. Method as claimed in claims 1-4, c h a r a c -
t e r i s e d in that the solid surface is an activated
organic polymer surface, and that the unsubstituted poly-
ethylene imine has a molecular weight of 10,000-100,000.

6. A solid surface having a biopolymer-repellent
30 hydrophilic outer layer consisting of nonionic polymer
chains covalently bonded at one end to a polyethylene
imine which in turn is bonded to the solid surface via
anionic groups capable for reacting with amino groups in
the polyethylene imine, c h a r a c t e r i s e d in
35 that the weight of the nonionic polymer chains is at least
50% of the weight of the polyethylene imine.

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7. Surface as claimed in claim 6, characterised in that the nonionic polymer chains are polyalkylene glycol chains deriving from ethylene oxide or ethylene oxide and polypropylene oxide, butylene oxide
5 and/or tetrahydrofuran distributed randomly or in blocks.

8. Surface as claimed in claim 7, characterised in that the nonionic polymer chains are derived from nonionic water-soluble cellulose ethers.

9. Surface as claimed in claim 6, characterised in that the solid surface is an active
10 organic polymer surface, and that the polyethylene imine has a molecular weight of 1,000-100,000.

10. Agent for carrying the method as claimed in claim 1 into effect, characterised in that
15 it is a polyethylene imine substituted by nonionic hydrophilic polymer chains, the amount by weight of the polymer chains being at least 50% of the unsubstituted polyethylene imine.

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INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 91/00702

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: C 08 J 7/12, 7/04 // A 61 L 33/00														
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%; border: 1px solid black; text-align: left;">Classification System</th> <th style="width: 80%; border: 1px solid black; text-align: left;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; padding: 5px;">IPC5</td> <td style="border: 1px solid black; padding: 5px;">C 08 J; A 61 L</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched⁸</div> <p>SE,DK,FI,NO classes as above</p>			Classification System	Classification Symbols	IPC5	C 08 J; A 61 L								
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III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; text-align: left;">Category *</th> <th style="width: 60%; text-align: left;">Citation of Document,¹¹ with indication, where appropriate, of the relevant passages¹²</th> <th style="width: 30%; text-align: left;">Relevant to Claim No.¹³</th> </tr> </thead> <tbody> <tr> <td style="vertical-align: top;">A</td> <td>PROGR COLLOID & POLYMER SCI, 74(1987) E KISS et al: "Surface grafting of polyethyleneoxide optimized by means of ESCA", page 113 - page 119 --</td> <td style="vertical-align: top;">1-10</td> </tr> <tr> <td style="vertical-align: top;">A</td> <td>EP, A1, 0341498 (E.I. DU PONT DE NEMOURS AND COMPANY) 15 November 1989, see the whole document --</td> <td style="vertical-align: top;">1-10</td> </tr> <tr> <td style="vertical-align: top;">A</td> <td>Chemical Abstracts, volume 114, no. 14, 8 April 1991, (Columbus, Ohio, US), Per Stenius et al: "Nonionic hydrophilic surfaces: adsorption and interactions ", see page 473, abstract 129778m, & Croat. Chem. Acta 1990, 63(3), 501-516 --</td> <td style="vertical-align: top;">1-10</td> </tr> </tbody> </table>			Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	A	PROGR COLLOID & POLYMER SCI, 74(1987) E KISS et al: "Surface grafting of polyethyleneoxide optimized by means of ESCA", page 113 - page 119 --	1-10	A	EP, A1, 0341498 (E.I. DU PONT DE NEMOURS AND COMPANY) 15 November 1989, see the whole document --	1-10	A	Chemical Abstracts, volume 114, no. 14, 8 April 1991, (Columbus, Ohio, US), Per Stenius et al: "Nonionic hydrophilic surfaces: adsorption and interactions ", see page 473, abstract 129778m, & Croat. Chem. Acta 1990, 63(3), 501-516 --	1-10
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents:¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>														
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border: 1px solid black; padding: 5px;"> Date of the Actual Completion of the International Search 28th January 1992 </td> <td style="width: 50%; border: 1px solid black; padding: 5px;"> Date of Mailing of this International Search Report 1992 -02- 03 </td> </tr> <tr> <td style="border: 1px solid black; padding: 5px;"> International Searching Authority SWEDISH PATENT OFFICE </td> <td style="border: 1px solid black; padding: 5px;"> Signature of Authorized Officer <i>Sofia Nikolopoulou</i> Sofia Nikolopoulou </td> </tr> </table>			Date of the Actual Completion of the International Search 28th January 1992	Date of Mailing of this International Search Report 1992 -02- 03	International Searching Authority SWEDISH PATENT OFFICE	Signature of Authorized Officer <i>Sofia Nikolopoulou</i> Sofia Nikolopoulou								
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International Searching Authority SWEDISH PATENT OFFICE	Signature of Authorized Officer <i>Sofia Nikolopoulou</i> Sofia Nikolopoulou													

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category "	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	<p>Dialog Information Services, File 350, World Patent Index 63-80, Dialog accession no. 2512387, TOYBO K: "Polyolefin film having improved adhesion propertie - having coating of polyethyleneimine and polyepoxy cpd", JP 55037338, A, 800315, 8017 (Basic)</p> <p>-----</p>	1-10

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.PCT/SE 91/00702

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 30/11/91
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0341498	89-11-15	JP-A- 2043947 US-A- 4952519	90-02-14 90-08-28

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